Stanley Cohen 11/9/77

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Donald S. Fredrickson, M.D.,
Director,
National Institute of Health
Building 1, Room 124
BETHESDA, Md. 20014

Dear Don:

I believe the proposed revisions of the NIH Guidelines on recombinant DNA research, as recently published in the Federal Register, represents a substantial improvement over the earlier version of the Guidelines. However, I do have a few comments and suggestions about the revisions.

(1) Definition of Recombinant DNA. There is an ambiguity in the proposed definition of "novel recombinant DNA." In order for a recombinant DNA molecule not to be considered novel, the Guidelines require that all of its components be derived from species that are known to exchange chromosomal DNA by natural physiological processes. This suggests that even if plasmids can be exchanged between two bacterial species by physiological processes, recombination of plasmid DNA segments in vitro is considered "novel" if exchange of chromosomal DNA has not also been demonstrated. However, further down in the same paragraph there is the statement that "all recombinant DNA molecules formed from any combination of DNAs will not be considered novel when all components are derived from genomes known to replicate within the organism used to propagate the recombinant DNA." This sentence implies that plasmid recombinants would not be considered novel if the plasmids can be exchanged, regardless of whether exchange of chromosomal DNA can occur. I think this apparent contradiction needs to be resolved.

My suggestion would be to define "novel recombinant DNAs" as follows: "molecules that consist of segments of any DNA from different species that are not known to exchange DNA by natural physiological processes." I know of no scientific basis for suggesting that organisms known to exchange extrachromosomal genetic information cannot also transfer chromosomal genes. Transduction and intracellular recombination between chromosomal and extrachromosomal DNA are well recognized biological processes.

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The modification I suggest would remove any ambiguity and would eliminate the apparent contradiction with the statement contained later in the paragraph 1 of the "Introduction" section of the Guidelines.

(2) Other host vector systems. Many of the practical benefits of recombinant DNA will depend on the ability to manipulate genes within organisms that produce medically and biologically important products such as antibiotics. The development of cloning systems in such species is, in my opinion, of great value. Yet, in revising the guidelines, the authors of the section on "Other host vector systems" seem to have given little consideration to the importance of being able to manipulate the genes of such species per se, and have instead dealt with other host vector systems simply as possible alternatives to E. coli for the cloning of genes of higher organisms.

The revised guidelines state that cloning in host vector systems other than E. coli must use strains that have "low potential for survival in their natural environment," regardless of the lack of pathogenicity of the species or the nature of the natural environment of that species. This appears to be a direct transfer with little additional thought of considerations pertaining to the use of E. coli K12 for recombinant DNA experiments. I think it is important to recall that the "low survival" attributes of E. coli K12 were initially put forth as a response to those who had concerns about using a bacterial strain that they believed might inhabit the human intestine. At that time, critics proposed that only bacterial species unassociated ecologically with humans or with domestic animals should be used. Subsequently, data has been presented to indicate that E. coli K12 has little, if any ability to colonize the human gastrointestinal tract. Somehow, in the course of events, the argument that it is safe to use E. coli K12 as a host because it does not colonize the human intestine, has been distorted. The statement is now being made that E. coli K12 should be the only approved host, and that other bacterial species -- no matter how non-pathogenic and remote from human ecology -- must not be used unless it is specifically demonstrated that the recipients have a low potential for survival in their natural environment (whatever that environment may be).

I believe that this new requirement will seriously impede and perhaps entirely cripple work aimed at using a variety of non-pathogenic bacteria as hosts in experiments aimed at improving the quality or quantity of biological products made by those bacteria. For example, an experiment involving the introduction of genes by recombinant DNA methods from Streptomyces coelicolor to Streptomyces lividans, in order to design a more effective antibiotic, could simply not be done in the wild type antibiotic-producing strain at any level of containment whatsoever.

These two organisms, although both closely related members of the genus Streptomyces, have not been shown to exchange DNA by natural physiological processes, (although DNA can be exchanged by experimentally induced cell fusion) and gene exchange by in vitro methods would come under the NIH Guidelines. Since the wild type recipient strain Streptomyces lividans does not have "low potential for survival in its natural environment", it could not even be classified as an HV1 system. Thus, even though there would probably be unanimous agreement by scientists that the experiment I've described above poses virtually no risk whatsoever, the experiment could not be carried out, even under high levels of physical containment, under the proposed Guidelines. By defining an HV 1 system in an inappropriate way, the authors of the revised Guidelines effectively have included recombinant DNA experiments with wild type non-E.coli hosts in the list of "prohibited experiments". Certainly the advisory committee could not have intended to place a Streptomyces coelicolor-Streptomyces lividans recombinant DNA molecule on the "too dangerous to be done" list, while at the same time permitting the cloning of DNA from a wide variety of animal cell and viral sources in E. coli K12 under minimal to moderate containment conditions. Yet, this is precisely the inevitable effect of the wording currently used. I urgently ask that you resolve this serious inconsistency prior to approval of the revised Guidelines.

I suggest that the problem can be resolved by simply having the Guidelines state that "wild type isolets of any bacterial species not known to be pathogenic to humans, to domestic animals, or to agriculturally important plants may be used as an HV1 host-vector system, provided that all components of recombinant DNA molecules introduced into such a host vector system, are derived from other prokaryotic organisms within Etiologic Agent Class 1. Use of a non-E.coli HV system for the propagation of genes derived from organisms other than those included in Etiologic Agent Class 1, or which are derived from eukaryotic organisms, shall be subject to certification as described in the Guidelines."

I believe this modification will provide adequate safety while still enabling work of great importance and minimal hazard to proceed.

(3) Levels of biological and physical containment for certain experiments. In most instances, the investigator is given the option of using either P2 + EK2 containment or P3 + EK1. However, in the section dealing with shotgun experiments from mammals other than primates (page 49601 of the Federal Register, September 27, 1977), and in the experiments dealing with DNA from birds, there is no option of trading a higher level of physical containment for a lower level of biological containment. Depending on the gene to be cloned, there may be legitimate and compelling experimental reasons for an investigator to wish to use P3 + EK1 for such experiments, rather than P2 + EK2. For example, the approved EK2 host (or hosts) may not be suitable for detection of the gene to be cloned (e.g. the thy A mutation of X1776 prevents detection of expression of mammalian genes coding for the enzyme dihydrofolate reductase, since the host bacterial strain is already resistant to trimethoprim and methotrexate).

I believe that, for consistency, the Guidelines should give investigators the option to trade a level of physical containment for a level of biological containment in these categories, as they have done in others. If the principle of making such a trade is valid, it should be applied across the board.

(4) The institutional committee to be established in connection with recombinant DNA should be called an institutional biosafety committee, rather than a "~~biohazards" committee. It seems~~ to me that the purpose of the institutional committee is to ensure the biosafety of recombinant DNA experimentation, whether or not biohazards are actually involved. Since the introductory section clearly points out that "to date, no known hazardous organism has been produced in this work," the term "biosafety committee" would seem to be more appropriate and would more accurately describe the responsibility of the committee. Continued use of the term "biohazards committee" helps to foster fears which, in my opinion, do not appear to be warranted.

biohazard to bio safety

I hope that these comments and suggestions will be useful to you and to the Advisory Committee in preparing the final version of the revised Recombinant DNA Guidelines.

Sincerely yours,

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Professor of Medicine and
Professor of Genetics

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